THE RESISTANCE AND ACCLIMATIZATION OF MARINE FISHES TO TEMPERATURE CHANGES. II. EXPERIMENTS WITH FUNDULUS AND ATHERINOPS [1]

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INTRODUCTION

In an earlier paper (Doudoroff, 1942) a detailed study of the resistance and acclimatization of the marine fish *Girella nigricans* to extreme high and low temperatures was presented. Some parallel experiments on the temperature tolerance of the relatively hardy species *Fundulus parvipinnis* and of the very delicate *Atherinops affinis* are reported here. While no attempt has been made to investigate fully the causes of death on chilling, some observations are recorded which bear on this problem.

The literature on the resistance and acclimatization of fishes and of other animals to low and high temperatures has been reviewed in the earlier publication. Of papers which have come more recently to the author's attention, those of Brett (1941, 1944) and of Fry, Brett and Clawson (1942) on the lethal limits of temperature for various fresh-water fishes must be mentioned. The importance of acclimatization as a factor to be considered in delimiting the temperature tolerance of these fishes was demonstrated fully. Certain early assumptions relative to the mortality rate at lethal temperatures (namely, that death occurs almost always within 12 or 14 hours even at low temperatures) and relative to the rate of acclimatization to low temperatures were not fully substantiated and are not entirely in accord with the author's observations on marine fishes.

The survival of some hardy fishes at very low, sub-zero temperatures in a state of seemingly suspended animation formerly had received more attention than death at low temperatures well above 0° C., and frequently has been termed "anabiosis" (Borodin, 1934; Schmidt, Platonov and Person, 1936). This term is misleading. The seemingly lifeless condition of supercooled and superficially frozen fish may be due to reversible injury to the central nervous system. The sensitive automatic mechanism of the heart also may be affected (Battle, 1926). There is no evidence that tissue respiration and all other vital functions are arrested (see also Kalabuchow, 1935), nor that the so-called anabiotic state can persist for a very long time. Therefore, this state may not differ fundamentally from chill-coma at above-zero temperatures and even may be closely related to heat-coma. The low metabolic rate at the lower temperatures and the hardiness of the species in which "anabiosis" has been observed account for the fact that the coma, with attendant respiratory and possibly circulatory disturbances, can be more prolonged at sub-zero temperatures.

Weigmann (1936) has contended that the death of fish which he super-cooled to very low temperatures was caused by respiratory disturbances. These he believed to be due to a direct effect of cooling upon the respiratory center of the brain, although other possibilities also were suggested. The observation that the animals could be revived, after return to normal temperatures, by the administration of artificial respiration was advanced as one of several arguments in support of the opinion that some respiratory disturbance was the cause of death. It will be shown that other factors can play an important role in the causation of death by chilling.

Methods and Material

The methods and equipment used in the evaluation of temperature tolerance were those employed in the experiments with Girella (Doudoroff, 1942). Briefly, the measures of relative tolerance adopted are the lower and upper median tolerance limits (T_m), defined as the low and high temperatures, respectively, which are lethal to just 50 per cent of the experimental animals in a given specified period of exposure. These values are estimated, when necessary, by interpolation, being then based on observed percentages of specimens surviving at two successive, constant test temperatures, usually $1°$ C. apart, one of which is lethal to more than half, and the other to less than half of the animals tested. Estimation of median tolerance limits for a series of time intervals permits verification of the individual values and frequently evaluation of the ultimate median tolerance limit, that is, the temperature which is tolerated indefinitely by 50 per cent of the animals.

Usually the fish were acclimatized and tested in constant-temperature aquaria supplied with running sea water, but in experiments in which the salinity of the medium was varied, jars with standing, continuously aerated water were used. Unless otherwise denoted here, the medium was sea water.

Fundulus parvipinnis Girard inhabits shallow and enclosed bays, sloughs and estuaries. Adults of both sexes, taken in Mission Bay near San Diego, were used. Average and minimal temperatures in the habitat of the species in this locality probably do not differ greatly from those in open water at the Scripps Institution of Oceanography nearby (Doudoroff, 1942). Maximal temperatures to which Fundulus may be exposed for brief periods in summer are considerably higher, however, than those recorded in open water, temperatures of $30°$ C. or higher probably being not uncommon during low tide in some places which are frequented by these active fishes.

Young *Atherinops affinis* (Ayres) were taken near the Scripps Institution at La Jolla, where, like Girella, they are common in open water near the shore. This species also is common in bays and estuaries, but, unlike Fundulus, it apparently does not frequent very shallow inlets and sloughs of the coastal marshes, where temperatures much higher than those in open water occur frequently. Thus, Atherinops probably lives under approximately the same temperature conditions as Girella in the same general locality. According to Jordan, Evermann and Clark (1930), the range of distribution of Atherinops extends along the "coast of California and

north to Oregon," *i.e.*, further north than that of Girella, while that of **Fundulus** extends "from Point Conception" (actually it extends somewhat further north) "southward to Lower California."

Comparative tests showed clearly that Atherinops is less resistant and **Fundu**lus is more resistant than Girella both to reduced salinity (dilution) of the medium and to asphyxiation (in tightly stoppered bottles in which the three forms were confined together). In each of these tests, which were performed with groups of specimens of equal average weight, all specimens of each of the less resistant species succumbed long before any of the specimens of the next more resistant species.

Only a relatively small number of the delicate and active Atherinops could be brought to the laboratory and kept for long periods in good health. Fundulus, although hardy, is subject to disastrous epidemic diseases in the laboratory (Wells and ZoBell, 1934) and often is heavily parasitized. Care was taken to select for experimentation only healthy specimens without parasitic isopods on the gills.

<div align="center">RESULTS</div>

Effects of extreme temperatures

Fundulus and Atherinops were affected by exposure to extreme high and low temperatures much as were Girella (Doudoroff, 1942), but the following important differences were noted.

If returned to a normal temperature within a few minutes after the onset of heat-coma, Fundulus, unlike Girella and Atherinops, often showed partial, temporary recovery, and sometimes complete recovery.[3] The administration of artificial respiration, by passing a stream of water through the mouth and over the gills, favored the recovery.

At slowly lethal temperatures primary chill-coma, that is, temporary cessation of all movements almost immediately following transfer to a low temperature, often was more prolonged in Fundulus than in Girella. Fundulus sometimes showed spontaneous, temporary recovery after remaining in a state of complete primary coma for more than one hour.[4] Specimens which failed to resume respiratory movements within two hours after cooling never moved thereafter at the low temperature. If returned to a normal temperature, these fishes were able to recover after longer periods, but recovery after three hours was observed only in one instance. However, recovery was obtained by the administration of artificial respiration together with warming when warming alone was not sufficient. This is illustrated by the following experimental results. Fourteen specimens, which had been held at 20° C., were exposed to 2° C., at which temperature none recovered from primary coma. After three hours the fishes were returned to 20° C., and artificial respiration was administered to one half of the specimens. All of the latter specimens resumed respiratory movements, and all but one recovered completely and were alive after three days. The seven specimens to which artificial respiration was not administered showed no recovery whatsoever.

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[3] Recovery has been obtained with other hardy fishes, e.g., *Gillichthys mirabilis* (Sumner and Doudoroff, 1938). Injury to the integument and paralysis of body muscles sometimes were noted in Fundulus which had recoverd partially after exposure to very extreme high temperatures. At these temperatures bleeding of the gills was observed occasionally.

[4] Such temporary recovery after prolonged primary chill-coma was observed in tests with other hardy species, e.g., *Leptocottus armatus* Girard.

If spontaneous recovery from primary chill-coma occurred at the low temperature at which the coma was produced, Fundulus always survived at this temperature for more than 12 hours before the onset of permanent, secondary chill-coma. For example, of 24 specimens which were transferred from 20° to 3° in a preliminary test only 14 recovered from primary coma, yet all of these survived for 12 hours, and 9 were still alive after 24 hours. The surviving specimens righted themselves and appeared normal. Later, however, they became seemingly stiff and emaciated, their eyes becoming deeply sunken. This appearance suggested osmotic dehydration of the tissues. Secondary chill-coma followed. If warmed thereafter, Fundulus sometimes showed brief activity, but complete recovery was unusual.

In Atherinops temporary recovery after complete primary chill-coma was observed rarely. Violent paroxysms, loss of equilibrium and partial inactivation often occurred after cooling and could be followed by recovery, but if movements ceased entirely for more than a few minutes, the coma was always permanent, and after one hour this species never recovered even on transfer to a normal temperature.

Permanent cessation of respiratory and other movements, either spontaneous or induced by mechanical stimulation, was regarded as the onset of death or endpoint of survival, as in the experiments with Girella. With Atherinops, as with Girella, determinations could be made of lower median tolerance limits for exposure periods of one hour or more. Inasmuch as Fundulus sometimes recovered from primary chill-coma after periods longer than one hour, the shortest exposure period adopted for cold-tolerance tests with this species was three hours.

Time-temperature relation of cold-tolerance in Fundulus

Fundulus collected in late summer and stocked at current sea water temperatures of 19° to 21° were soon transferred to constant-temperature tanks at 14°, 20° and 28°. Many of the specimens held at 14° and most of those at 28° died of disease. The relatively healthy 20°-conditioned fish were used in cold-tolerance tests after a sojourn of 30 days or longer in the constant-temperature tank. The experimental results are presented in Table I. Since all specimens which survived for 3 hours survived also for 12 hours, the data for these exposure periods appear in a single column.

TABLE I

Per cent survival of 20°-conditioned Fundulus at various low temperatures and the estimated lower median tolerance limits (T_m) in relation to time (duration) of exposure to the test temperatures

Date 1940	No. of fish and ratio ♂ : ♀	Mean length (cm.)	Test temp. (° C.)	Per cent surviving after							
				3 to 12 hours	1 day	2 days	3 days	4 days	5 days	6 days	7 days
8/28	12 (9:3)	6.8	7°	100%	100%	100%	100%	100%	100%	100%	100%
9/4	14 (10:4)	6.7	6°	100	100	100	100	100	93	86	86
9/11	12 (9:3)	6.7	5°	100	100	100	92	67	25	0	0
9/22	12 (8:4)	6.7	4°	100	100	100	83	25	0	0	0
9/18	16 (12:4)	6.7	3°	87	81	50	0	0	0	0	0
9/27	14 (10:4)	6.7	2°	0	0	0	0	0	0	0	0
		T_m:		2.6°	2.6°	3.0°	3.6°	4.6°	5.4°	5.6°	5.6°

The lower median tolerance limits are plotted against exposure time in Figure 1. The sigmoid time-temperature curve of cold-tolerance shown differs strikingly from curves obtained with Girella (Doudoroff, 1942, Fig. 1), which showed most of the rise of T_m with increasing time of exposure occurring during the first day or two. The ultimate lower median tolerance limit for the Fundulus apparently is close to 5.6°.
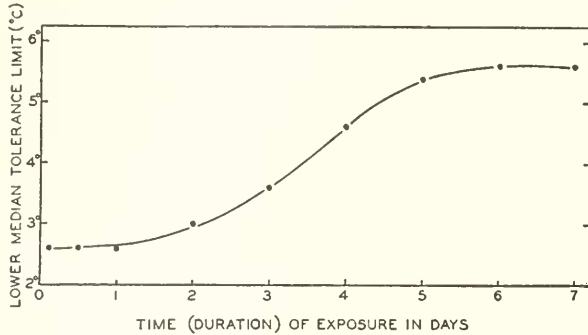

FIGURE 1. Time-temperature curve of cold-tolerance of 20°-conditioned Fundulus: Lower median tolerance limits in relation to the time (duration) of exposure to the test temperatures in sea water. Data from Table I.

Comparison of the data with the results of some earlier preliminary experiments and of later tests revealed a small progressive increase of the resistance of the 20°-conditioned Fundulus to cold, and especially to the less extreme low temperatures. The cause has not been established, but the change may have been seasonal or related to the sexual cycle.

Changes of water content at lethal low temperatures and the influence of dilution of the medium and acclimatization on cold-resistance in Fundulus

The loss of tissue water through osmosis by Fundulus which were dying in sea water at slowly lethal low temperatures was inferred from the appearance of the animals. Data presented in Table II, which shows the relative water content (*i.e.,* weight lost on desiccation to constant weight at 100° per unit of the dry weight) for groups of specimens differing in their history, support this inference. The experimental animals which had been exposed to low test temperatures were removed for weighing when they were seemingly near death, but still respiring and responding to stimulation. The results obtained with 14°-conditioned specimens indicate an average loss of more than 23 per cent of the normal water content at the lethal low temperatures (1.0°–1.6°) in sea water, but no loss in diluted (45 per cent) sea water. The specimens tested in the diluted water did not appear dehydrated and were still quite lively when they were removed, although they had been exposed to the low temperature (1.6°) about twice as long as the specimens tested in sea water. The relatively large 20°-conditioned specimens apparently lost, on the average, 14 per cent of their initial water content at the lethal test temperature (3.0°) in sea water.

Osmotic work must be done constantly by marine fishes in their normal medium. Dehydration can result from disturbances of the osmoregulative functions, but

TABLE II

Water content of Fundulus after exposure to slowly lethal low temperatures in sea water (SW) and in diluted sea water (45% SW), and that of control specimens taken directly from sea water at the initial acclimatization temperatures. All of the experimental specimens were still alive when removed from the test aquaria. The water content is expressed as units of weight of water per unit of dry weight. The mean values indicated for fish with similar history are weighted according to the number of specimens.

Acclim. temp. (° C.)	Test temp. (° C.)	Days at test temp.	Test medium	Number and sex of fish	Wet weight (grams)	Dry weight (grams)	Wet wt. − Dry wt. / Dry wt.
14°	1.0°	2	SW	1 ♂	3.64	1.03	2.53
14°	1.6°	2	SW	1 ♂	5.06	1.48	2.42
14°	1.6°	2	SW	1 ♂	4.51	1.38	2.27
14°	1.6°	2	SW	2 ♂	7.55	2.25	2.36
					Weighted mean:		2.39
14°	1.6°	3½	45% SW	2 ♂	9.84	2.45	3.02
14°	1.6°	4	45% SW	5 ♂	19.29	4.57	3.22
					Weighted mean:		3.16
14°		Control		1 ♂	3.51	0.86	3.08
14°		Control		1 ♂	3.93	0.95	3.14
14°		Control		1 ♂	4.06	0.97	3.19
14°		Control		2 ♂	9.91	2.43	3.08
					Weighted mean:		3.11
20°	3.0°	1½	SW	1 ♀	6.46	1.75	2.69
20°	3.0°	2½	SW	1 ♂, 1 ♀	13.63	4.02	2.39
20°	3.0°	3	SW	2 ♂	14.65	4.32	2.39
					Weighted mean:		2.45
20°		Control		1 ♂	8.62	2.13	3.05
20°		Control		1 ♂, 1 ♀	15.61	4.08	2.83
20°		Control		1 ♂, 1 ♀	16.04	4.25	2.77
20°		Control		1 ♂, 1 ♀	11.60	3.01	2.85
					Weighted mean:		2.85

obviously can be prevented by sufficient dilution of the medium. *Fundulus parvipinnis* probably occurs only in salt water, but it can live in fresh water. The normal osmotic pressure of the blood of this species was not known. From available cryoscopic data for other marine and euryhaline fishes (Dakin, 1912, 1935) it was concluded, however, that 45 per cent sea water probably is roughly isosmotic with the blood of Fundulus, or only slightly hypertonic. The results of comparable tests of the cold-resistance of specimens acclimatized to 20° and to 14° and tested in sea water and in 45 per cent sea water (prepared by dilution with water distilled in glass) are presented in Table III. The specimens tested in the diluted water were held in this medium for one day before the test.

Table III

Per cent survival of Fundulus at various low test temperatures and the estim: zted lower median tolerance limits (T_m) in relation to temperature of previous acclimatization, to dilution of the medium (SW = sea water) and to time (duration) of exposure to the test temperatures

Date 1940	Acclim. temp. (°C.)	Test medium	No. of fish and ratio ♂:♀	Mean length (cm.)	Test temp. (°C.)	Per cent surviving after											
						3 to 12 hours	1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days	11 days
10/6	20°	SW	8 (5:3)	6.7	4.0°	100%	100%	100%	100%	87%	37%	0%	0%	0%	0%	0%	0%
10/18	20°	SW	8 (4:4)	6.7	3.0°	100	100	100	87	0	0	0	0	0	0	0	0
10/30	20°	SW	8 (5:3)	6.7	2.0°	37	37	12	0	0	0	0	0	0	0	0	0
					T_m :	2.2°	2.2°	2.4°	2.6°	3.6°							
10/6	20°	45% SW	8 (5:3)	6.7	4.0°	100%	100%	100%	100%	100%	100%	100%	75%	62%	37%	12%	0%
10/18	20°	45% SW	8 (4:4)	6.7	3.0°	100	100	100	100	100	100	100	37	12	0	0	0
10/30	20°	45% SW	8 (5:3)	6.7	2.0°	37	37	25	12	0	0	0	0	0	0	0	0
					T_m :	2.2°	2.2°	2.3°	2.4°	2.5°	2.5°	2.5°	3.3°	3.8°			
11/12	14°	SW	8 (5:3)	6.7	1.6°	100%	100%	87%	0%	0%							
11/4	14°	SW	8 (5:3)	6.7	1.0°	87	75	25	12	0							
11/8	14°	SW	8 (5:3)	6.7	0.4°	12	12	0	0	0							
					T_m :	0.7°	0.8°	1.2°									
11/12	14°	45% SW	8 (5:3)	6.7	1.6°	100%	100%	100%	100%	75%							

? Test discontinued.

At the rapidly lethal temperature of 2° there was no significant difference between the mortality rates of 20°-conditioned specimens in the two media, five of the eight fish in each group having failed to recover from primary chill-coma. At the less extreme low temperatures of 3° and 4°, on the other hand, all the specimens succumbed in sea water before any succumbed in the diluted water. If the mid-point of the 24-hour interval during which each specimen succumbed is taken as the time of its death, the mean survival times at the latter two temperatures are found to be 3.4 days and 4.75 days, respectively, in sea water, while in the diluted water they are 7.0 days and 8.4 days, respectively. The difference between the means for the two media at each of these slowly lethal temperatures is certainly significant, the probability being 0.9999 + .[5] Accordingly, T_m values for moderately long exposure periods only were clearly influenced by the salinity of the water. The results obtained with the 14°-conditioned specimens, which had been held at 14° for 83 to 91 days, likewise indicate clearly an influence of water salinity upon the mortality rate at a slowly lethal low temperature (1.6°), and also an influence of acclimatization upon cold-tolerance.[6]

Resistance and acclimatization of Fundulus to heat

Fundulus used in the heat-tolerance experiments were acclimatized in the three constant-temperature tanks (at 20° for 30 to 51 days, at 28° for 57 to 63 days, and

TABLE IV

Per cent survival of Fundulus at various high temperatures and the estimated upper median tolerance limits (T_m) in relation to temperature of previous acclimatization and to time (duration) of exposure to the test temperatures

Date 1940	Acclim. temp. (° C.)	No. of fish and ratio ♂: ♀	Mean length (cm)	Test temp. (° C.)	Per cent surviving after										
					0.5 hr.	1 hr.	3 hrs.	6 hrs.	12 hrs.	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.	144 hrs.
9/30	28°	12 (5:7)	6.8	40°	33%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
9/30	28°	13 (5:8)	6.8	39°	100	69	0	0	0	0	0	0	0	0	0
9/27	28°	13 (5:8)	6.7	38°	100	100	100	31	0	0	0	0	0	0	0
9/25	28°	12 (5:7)	6.7	37°	100	100	100	100	100	42	0	0	0	0	0
10/1	28°	13 (5:8)	6.8	36°	100	100	100	100	100	100	100	100	100	100	92
				T_m:	39.7°	39.3°	38.5°	37.7°	37.5°	36.9°	36.5°	36.5°	36.5°	36.5°	36.5°
9/18	20°	12 (8:4)	6.7	37°	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
9/14	20°	13 (9:4)	6.7	36°	54	54	38	38	23	8	0	0	0	0	0
9/4	20°	13 (9:4)	6.6	35°	100	100	100	100	100	100	85	54	38	31	31
8/28	20°	13 (9:4)	6.6	34°	100	100	100	100	100	100	100	100	92	85	62
				T_m:	36.1°	36.1°	35.8°	35.8°	35.7°	35.5°	35.4°	35.1°	34.8°	34.6°	34.4°
10/10	14°	12 (6:6)	6.7	34°	42%	25%	0%	0%	0%	0%	0%	0%	0%	0%	0%
10/11	14°	12 (6:6)	6.7	33°	100	100	33	8	8	8	8	8	8	8	8
10/22	14°	13 (7:6)	6.7	32°	100	100	92	77	77	69	69	69	69	69	69
				T_m:	33.9°	33.7°	32.7°	32.4°	32.4°	32.3°	32.3°	32.3°	32.3°	32.3°	32.3°

[5] For the method of computing probabilities see Tippett (1931).

[6] Fundulus which had been acclimatized to 28° and then held for 7 days at 36° died within 24 hours after subsequent cooling to 15°. Similar observations have been recorded by Wells (1935).

at 14° for 58 to 70 days) together with those used in the cold-tolerance tests. The experimental data are presented in Table IV.

The time-temperature relationship of heat-tolerance of 28°-conditioned Fundulus is somewhat similar to that observed with 20°-conditioned Girella, the values of T_m for exposure periods of 0.5 to 48 hours tending to fall along a straight line when plotted against the logarithm of the exposure time (Fig. 2). The 48-hour
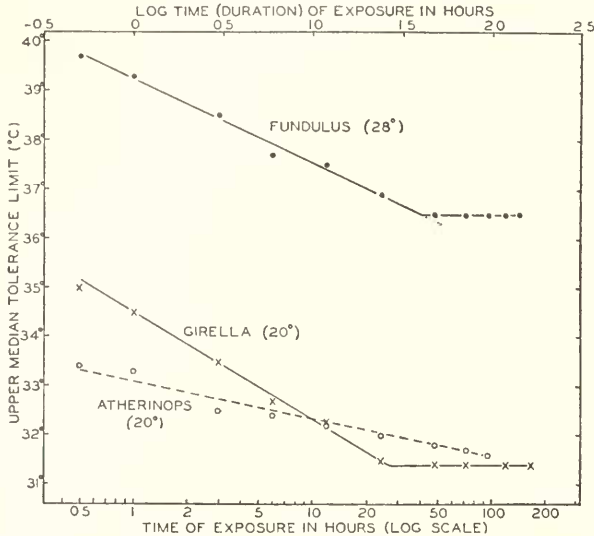

FIGURE 2. Time-temperature curves of heat-tolerance of 28°-conditioned Fundulus, 20°-conditioned Atherinops and 20°-conditioned Girella: Upper median tolerance limits in relation to the logarithm of the time (duration) of exposure to the test temperatures. Data from Tables IV and V and from Doudoroff (1942), Table II.

T_m, or 36.5°, apparently is also the ultimate median tolerance limit. The relationship obtained with 14°-conditioned Fundulus is not logarithmic and is not unlike that obtained with 12°-conditioned Girella. All deaths occurred during the first day of exposure, T_m remaining thereafter at 32.3°. The nearly straight-line relationship between T_m and duration of exposure obtained with Fundulus from the intermediate temperature of 20° is quite unlike any others which have been studied. No explanation can be suggested. Nevertheless, the influence of acclimatization upon resistance to heat is shown clearly by the data, and it is evident that Fundulus, unlike Girella, does not acquire nearly maximal heat-tolerance when it is acclimatized to 20°.

Resistance and acclimatization of Atherinops to heat and cold

Atherinops taken in October, when the surface temperature in their habitat had been close to 20° for some time, were held in the laboratory for three weeks at current sea water temperatures of 19° to 20°, and then in a constant-temperature tank at 20° for one week before tests were begun. The time-temperature relations of tolerance to heat and to cold were studied in the usual manner, but intervals of only 0.5° between successive test temperatures were used in the evaluation of heat-tolerance to ensure desired accuracy. The results are presented in Table V.

Great sensitiveness of this species to cooling is evident. While no simple time-

TABLE V

Per cent survival of 20°-conditioned Atherinops at various high and low temperatures and the estimated upper and lower median tolerance limits (T_m) in relation to time (duration) of exposure to the test temperatures

Date 1940	No. of fish	Mean length (cm.)	Test temp. (° C.)	Per cent surviving after								
				0.5 hr.	1 hr.	3 hrs.	6 hrs.	12 hrs.	24 hrs.	48 hrs.	72 hrs.	96 hrs.
11/6	12	6.1	33.5°	42%	17%	0%	0%	0%	0%	0%	0%	0%
11/5	14	6.1	33.0°	86	86	0	0	0	0	0	0	0
11/7	12	6.2	32.5°	100	100	50	33	0	0	0	0	0
11/2	12	6.2	32.0°	100	100	100	100	92	50	17	0	0
11/8	14	6.1	31.5°	100	100	100	100	100	100	86	71	57
		T_m (upper):		33.4°	33.3°	32.5°	32.4°	32.2°	32.0°	31.8°	31.7°	31.6°
11/28	18	6.2	11.0°		100%	100%	100%	100%	100%	100%	100%	100%
11/20	18	6.0	10.0°		100	100	100	94	94	44	22	22
11/16	16	6.1	9.0°		100	100	100	94	75	37	6	0
11/15	16	6.0	8.0°		100	75	25	0	0	0	0	0
11/14	16	6.1	7.0°		87	62	0	0	0	0	0	0
11/17	18	6.1	6.0°		56	28	0	0	0	0	0	0
11/16	16	6.2	5.0°		0	0	0	0	0	0	0	0
		T_m (lower):			5.9°	6.6°	8.3°	8.5°	8.7°	10.1°	10.4°	10.4°

temperature curve of cold-tolerance fits the data closely, the lower median tolerance limit rises progressively with increasing time of exposure for three days, whereupon an apparently constant value of 10.4° is reached. The upper median tolerance limits are plotted against the logarithm of the time of exposure in Figure 2, together with corresponding data for 20°-conditioned Girella and 28°-conditioned Fundulus.

Some Atherinops were held through the winter at current sea water temperatures, which were below 18°, and which averaged 16° during the last month. After subsequent acclimatization for about three weeks to a constant temperature of 18.5° these specimens were found to have lower and upper 24-hour median tolerance limits of 8.8° and 30.5°, respectively. While all the fish died within 24 hours after direct transfer to 31° in this experiment, after a two day sojourn at 30° all survived for 48 hours at 31°, and the majority survived at 32°, thus showing rapid acclimatization to heat. Rapid acclimatization to cold did not occur, but in another experiment with specimens which had been held at 20° and then acclimatized for more than two months to 14.5° and 25.5° a pronounced influence of long acclimatization upon cold-tolerance was demonstrated, the 24-hour lower median tolerance limits for the two lots being 7.6° and 13.5°, respectively.

DISCUSSION

Because of the nature and variability of the time-temperature relations of tolerance, prolonged tests often may be necessary in ecological studies of the resistance of fishes to cold and heat. The lower median tolerance limit for Fundulus, unlike

those for Girella and Atherinops, remained nearly constant during the first 48 hours of exposure (6 days in diluted sea water) and rose rapidly thereafter (Fig. 1). Limits of tolerance for uniform exposure periods even longer than 48 hours evidently may not be truly comparable and ecologically significant. Had extended observations not been made, the prolonged temporary recovery of Fundulus from primary chill-coma at slowly lethal low temperatures could have been mistaken for permanent recovery.

The time-temperature curves of heat-tolerance for 20°-conditioned Girella, 28°-conditioned Fundulus and 20°-conditioned Atherinops (Fig. 2) are somewhat similar, but they differ greatly with respect to their slope, the temperature coefficients, Q_{10}, of the velocity of heat injury in these fishes being about 4×10^4, 10^6 and 10^{13}, respectively.[7] In each of these instances Q_{10} is constant over the range of lethal temperatures used, but this coefficient evidently is not generally applicable. Bělehrádek (1935) listed temperature coefficients of heat injury for various organisms and tissues, the values of Q_{10} listed ranging from less than 10 to 58,000. Rapid acclimatization probably accounts for the unusually high values obtained with Fundulus and Atherinops.

The ranges of temperatures which were tolerated for three days by average 20°-conditioned specimens of the three species studied are 10.4° to 31.7° (21.3° range) for Atherinops, 8° to 31.4° (23.4° range) for Girella and 3.6° to 35.1° (31.5° range) for Fundulus. The 6-day tolerance limits for the Fundulus, which are 5.6° and 34.4° (28.8° range), are more nearly comparable with the 3-day values for the other two species. The magnitudes of the ranges are clearly related to the general hardiness of the species, and resistance to cold shows a parallel relationship.

The 20°-conditioned Atherinops were about as resistant to heat as Girella, but were more sensitive to cooling, their 72-hour lower median tolerance limit being only 1.3° below the lowest weekly average surface temperature recorded near the place of their capture.[8] While the temperature of 20° lies about half way between the extremes of temperature which may be encountered by Atherinops in this general locality, and is above the average surface temperature, the specimens taken from 20° withstood for four days a rise of temperature of 11.6° and a fall of only 9.6°. It is concluded that Atherinops, like Girella, certainly is not any more resistant to cooling than to warming. The importance of lethal cold as a possible limiting factor in the distribution of sensitive marine fishes, and of acclimatization as a factor in their dispersal, has now been amply demonstrated.[9] When all of the pertinent data are considered, it becomes evident that both Atherinops and Girella almost certainly cannot survive long at 0° even after very gradual cooling, the true range of thermal tolerance for Girella being smaller than that plotted by

[7] $Q_{10} = (K_1/K_2)^{10/(t_1 - t_2)}$, where K_1 and K_2 are velocity constants at temperatures t_1 and t_2, respectively, of which t_1 is the higher.

[8] Surface temperatures reported for the northern portion of the reputed range of distribution of *Atherinops affinis* (United State Weather Bureau, 1938) average 11° to 13°, corresponding winter averages being about 1° lower. However, the species may be represented in different portions of its range by physiologically different races, the resistance of which to cold may be inherently different.

[9] For data on the reactions of Fundulus and Atherinops, as well as of Girella, to temperature gradients, which also indicate a pronounced sensitiveness of these fishes to cooling, see Doudoroff (1938).

Brett (1944), who used the author's data, but plotted only 12-hour tolerance limits. The susceptibility of *Fundulus parvipinnis* to chilling is less pronounced, but its ability to withstand 0° indefinitely after slow cooling is questionable, and this species is correspondingly resistant to heat.

There is evidence that not only the speed, but also the cause of death after chilling can vary with the intensity of the cold. The rapid death of Fundulus at very extreme low temperatures was quite different from the delayed death which followed more or less prolonged temporary recovery from the initial effects of cooling at less extreme temperatures. Very brief recovery never was observed at intermediate lethal temperatures. Only at the slowly lethal temperatures was death preceded in sea water by evident dehydration of tissues and delayed significantly by dilution of the medium.

The observation that recovery from primary chill-coma could be induced by warming and the administration of artificial respiration when warming alone was not sufficient suggests respiratory inhibition (Weigmann, 1936) as a cause of death at rapidly lethal low temperatures. When respiration is not resumed within a short time after cooling, asphyxiation or carbon dioxide narcosis may prevent subsequent recovery. However, at low temperatures hardy fishes can survive for hours in deoxygenated water (Sumner and Doudoroff, 1938). It is not improbable that asphyxiation was of importance as a cause of death only after warming (i.e., return to a normal temperature) and the consequent increase of the rate of respiratory metabolism.

At slowly lethal low temperatures increasing distress, indicative of cumulative injury, was evident long before the permanent cessation of regular respiratory movements, and the behavior of Fundulus did not suggest asphyxiation. It appears that at these temperatures some osmoregulative functions, such as the ingestion of sea water or the active excretion of salts by the gills, which compensate for the passive exosmotic loss of water through the external membranes (Smith, 1930; Keys, 1933), were inhibited, and in the normal medium dehydration and death followed as a result. The failure of these functions probably was itself a consequence of cold injury to tissues involved in osmoregulation (e.g., of the gills, the central nervous system or the integument) and was not, therefore, the primary cause of injury. The fact that mortality, although it was much delayed, was not entirely prevented when dehydration was avoided by dilution of the medium indicates that other, less rapidly lethal disturbances occurred also. Osmoregulative failure is only one of a number of possible causative factors which should be taken into consideration in further studies of the phenomena of chilling.

SUMMARY

The resistance of the hardy marine fish *Fundulus parvipinnis* and of the delicate *Atherinops affinis* to low and high temperatures is examined in relation to temperatures of previous acclimatization and to temperature conditions in the natural habitats of the fishes.

The relationships between the time (duration) of exposure to test temperatures and the average (median) limits of temperature tolerance indicate the value of prolonged tests in the study of resistance to cold and heat.

Marked susceptibility of marine fishes to chilling (i.e., injury at non-freezing

low temperatures) and the influence of acclimatization upon temperature tolerance are confirmed.

The death of Fundulus at slowly lethal low temperatures, but not at rapidly lethal temperatures, is preceded in the normal medium (sea water) by evident dehydration of the tissues and is delayed by dilution of the medium. Osmoregulative failure is indicated as one of the causes of slow death at low temperatures in sea water, but not of rapid death at more extreme low temperatures, which may be caused partly by respiratory disturbances.

LITERATURE CITED

BATTLE, H. I., 1926. Effects of extreme temperatures on muscle and nerve tissue of fishes. *Trans. Roy. Soc. Canada*, **20**, Sec. V : 127–143.

BĚLEHRÁDEK, J., 1935. Temperature and living matter. *Protoplasma-Monographien,* **8** : Gebr. Bortraeger, Berlin.

BORODIN, N. A., 1934. The anabiosis or phenomenon of resuscitation of fishes after being frozen. *Zool. Jahrb., Abt. f. allg. Zool. u. Physiol.,* **53** : 313–341.

BRETT, J. R., 1941. Tempering versus acclimation in the planting of speckled trout. *Trans. Am. Fish. Soc.,* **70** : 397–403.

BRETT, J. R., 1944. Some lethal temperature relations of Algonquin Park fishes. *Publ. Ontario Fish. Res. Lab.,* No. 63. *Univ. of Toronto Studies, Biol Ser.,* No. 52.

DAKIN, W. J., 1912. Aquatic animals and their environment. The constitution of the external medium and its effect upon blood. *Internat. Rev. ges. Hydrobiol.,* **4** : 53–80.

DAKIN, W. J., 1935. The aquatic animal and its environment (Presidential address). *Proc. Linnean Soc. New South Wales,* **60** : vii–xxxii.

DOUDOROFF, P., 1938. Reactions of marine fishes to temperature gradients. *Biol. Bull.,* **75** : 494–509.

DOUDOROFF, P., 1942. The resistance and acclimatization of marine fishes to temperature changes. I. Experiments with Girella nigricans (Ayres). *Biol. Bull.,* **83** : 219–244.

FRY, F. E. J., J. R. BRETT, AND G. H. CLAWSON, 1942. Lethal limits of temperature for young goldfish. *Rev. canadienne de Biol.,* **1** : 50–56.

JORDAN, D. S., B. W. EVERMANN, AND H. W. CLARK, 1930. Check list of fishes and fish-like vertebrates of North and Middle America north of the northern boundary of Venezuela and Colombia. *Report of the U. S. Commissioner of Fisheries,* 1928, Part II, Doc. No. 1055.

KALABUCHOW, N. I., 1935. Anabiose bei Wirbeltieren und Insekten bei Temperaturen unter O (Zur Frage über die Unterkühlung und das Gefrieren der Tiere). *Zool. Jahrb., Abt. f. allg. Zool. u. Physiol.,* **55** : 47–64.

KEYS, A., 1933. The mechanism of adaptation to varying salinity in the common eel and the general problem of osmotic regulation in fishes. *Proc. Roy. Soc., B,* **112** : 184–199.

SCHMIDT, P. J., G. P. PLATONOV, AND S. A. PERSON, 1936. On the anabiosis of fishes in supercooled water. *C. R. Acad. Sci. U.R.S.S.,* **3** : 305–308.

SMITH, H. W., 1930. The absorption and excretion of water and salts by marine teleosts. *Am. Jour. Physiol.,* **93** : 480–505.

SUMNER, F. B., AND P. DOUDOROFF, 1938. Some experiments on temperature acclimatization and respiratory metabolism in fishes. *Biol. Bull.,* **74** : 403–429.

TIPPETT, L. H. C., 1931. *The methods of statistics.* London. Williams and Norgate.

UNITED STATES WEATHER BUREAU, 1938. Atlas of climatic charts of the oceans. W. B. **1247.**

WEIGMANN, R., 1936. Zur Kältebeständigkeit poikilothermer Tiere, Untersuchung an Schnecken und Fischen. *Biol. Zentralblatt,* **56** : 301–322.

WELLS, N. A., 1935. Variations in the respiratory metabolism of the Pacific killifish, Fundulus parvipinnis, due to size, season and continued constant temperature. *Physiol. Zool.,* **8** : 318–336.

WELLS, N. A., AND C. E. ZOBELL, 1934. Achromobacter ichthyodermis, n. sp., the etiological agent of an infectious dermatitis of certain marine fishes. *Proc. Nat. Acad. Sci.,* **20** : 123–126.